

Data Evaluation Record on the Reproductive Effects of Ethylenethiourea (ETU) on Northern Bobwhite Quail (*Colinus virginianus*)

PMRA Submission Number {.....}

EPA MRID Number 48819701

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	402789
	OECD Data Point	{.....}
	EPA MRID	48819701
	EPA Guideline	OPPTS 850.2300

Test material: Ethylenethiourea

Purity: 100%

Common name: Ethylenethiourea (ETU)

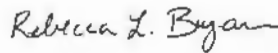
Chemical name: IUPAC: Not reported

CAS name:

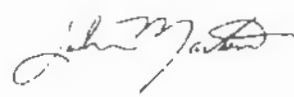
CAS No.: 96-45-7

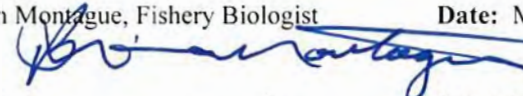
Synonyms: 2-imidazolidinethione; ETU

Primary Reviewer: Rebecca L. Bryan
Staff Scientist, CSS-Dynamac Corporation

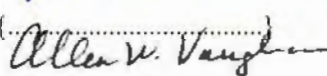
Signature: 
Date: 5/28/13

Secondary Reviewer: John Marton, Ph.D.
Environmental Scientist, CDM Smith

Signature: 
Date: 3/13/14

Primary Reviewer: Brian Montague, Fishery Biologist
{EPA/OECD/PMRA} 

Date: March 3, 2015

Secondary Reviewer(s): {.....}
{EPA/OECD/PMRA} 

Date: {.....}
04/16/15

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category	{.....}	[For PMRA]
EPA PC Code	600016	

Date Evaluation Completed: {dd-mm-yyyy}

CITATION: Temple, D.L., *et al.* 2012. Ethylenethiourea: A Reproduction Study with the Northern Bobwhite. Unpublished study performed by Wildlife International Ltd., Easton, MD. Laboratory Study No. 697-107. Study sponsored by EBDC/ETU Task Force, c/o McDermott, Will and Emery, Washington, D.C.

Study initiated August 1, 2011 and completed April 30, 2012. Biological portion: August 2, 2011 to January 20, 2012.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the reproductive effects of a pesticide on avian species. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY

The one-generation reproductive toxicity of Ethylenethiourea (ETU) to 16 pairs per level of 20-week old Northern Bobwhite quail (*Colinus virginianus*) was assessed over 20 weeks. Ethylenethiourea was administered to the birds in the diet at nominal concentrations of 0 (negative control), 20, 100, and 400 mg ai/kg diet. Mean-measured concentrations were 0 (control), 18.2, 100, and 381 mg ai/kg diet.

No mortalities occurred in the control or 381 mg ai/kg groups. Incidental mortalities included one 18.2 mg ai/kg group male bird found dead on Day 3 of Week 6, and one 100 mg ai/kg group female bird found dead on Day 6 of Week 20. No treatment-related signs of toxicity were observed in the control or treatment groups. No treatment-related findings were observed in the ≤ 100 mg ai/kg diet groups based on the gross necropsy results. At 381 mg ai/kg, the treatment-related finding of enlarged thyroids was observed for one male and four female adult birds. There were no treatment-related differences in feed consumption or in adult body weights at any treatment level. At 100 and 381 mg ai/kg diet, hatchling body weights were significantly ($p < 0.05$) decreased compared to controls. At 381 mg ai/kg diet, 14-day old survivor weight, 14-d hatchlings per egg set, hatchlings per live embryo, male weight gain, and female weight gain were significantly ($p < 0.05$) reduced compared to controls. No apparent treatment-related effects on reproduction or statistically-significant differences from the control were observed at any interval for the 18.2 or 100 mg ai/kg-diet levels. No significant treatment-related differences in egg shell thickness were observed between the control group and any of the treatment groups. The NOAEC was 18.2 mg ai/kg diet (mean-measured concentration) based on hatchling bodyweights.

This study is scientifically sound and will satisfy the FIFRA guideline requirement for a Northern Bobwhite quail (*Colinus virginianus*) reproductive toxicity study.

Results Synopsis

Test Organism Size/Age (mean Weight): 20 weeks old; 173 to 228 g (combined sexes)

NOAEC: 18.2 mg ai/kg (mean-measured)

LOAEC: 100 mg ai/kg (mean-measured)

Endpoints affected: 14-d hatchlings/eggs set, hatchlings/live embryo, hatchling weight, 14-d survivor weight, male weight gain, female weight gain

Most sensitive endpoint(s): Hatchling body weight

I. MATERIALS AND METHODS

GUIDELINE(S) FOLLOWED: U.S. EPA Pesticide Assessment Guidelines, §71-4, U.S. EPA Ecological Effects Test Guidelines OPPTS 850.2300, and OECD Test Guideline 206.

No deviations from U.S. EPA OPPTS Guideline No. 850.2300 were observed.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided. The study was conducted in compliance with the U.S. EPA GLP Standards (40 CFR part 160) with the exception of routine water and food contaminant screening analyses. However, the analyses were performed using laboratory standard operating procedures and standard U.S. EPA procedures.

A. MATERIALS:

1. Test Material: Ethylenethiourea (ETU)

Description: Solid

Lot No./Batch No. : XW7-102353-014

Purity: 100%

Stability of compound under test conditions: The Day 7 diet sample recoveries were 91-99% of Day 0 values.

Storage conditions of test chemicals: Ambient conditions.

Physicochemical properties of Ethylenethiourea.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

2. Test organism:

Table 1: Test organism

Parameter	Details	Remarks
		Criteria
Species (common and scientific names):	Northern Bobwhite quail (<i>Colinus virginianus</i>)	<i>Recommended species include a wild waterfowl species, preferably the mallard (Anas platyrhynchos) or an upland game species, preferably the Northern Bobwhite (Colinus virginianus)</i>
Age at Study Initiation:	20 weeks old	<i>Birds approaching their first breeding season should be used.</i>
Body Weight: (mean and range)	Overall (combined sexes, combined treatments) range of 173 to 228 g at study initiation. Adult body weight was measured every 2 weeks until week 8	<i>Body weights should be recorded at test initiation and at biweekly intervals up to week eight or up to the onset of egg laying and at termination.</i>
Source:	Molly Bryden, Rock Hall, MD	Birds were phenotypically indistinguishable from wild stock. <i>All birds should be from the same source.</i>

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: No range-finding study was reported.

b. Definitive Study:

Table 2: Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period:	2 weeks	
Conditions (same as test or not):	Not reported.	<i>Recommended observation period includes a 2-3 week health observation period prior to selection of birds for treatment. Generally, birds should be healthy without excess mortality. Feeding should be <u>ad libitum</u>, and sickness, injuries or mortality should be noted.</i>
Feeding:	Basal diet was provided daily, <i>ad libitum</i> .	
Health (any mortality observed):	Healthy birds were used in test.	
<u>Test duration</u>		
Pre-laying exposure:	10 weeks (including photo-stimulation period)	<u>Recommended pre-laying exposure duration:</u> <i>At least 10 weeks prior to the onset of egg-laying.</i> <u>Recommended exposure duration with egg-laying:</u> <i>At least 10 weeks.</i> <u>Recommended withdrawal period:</u> <i>If reduced reproduction is evident, a withdrawal period of up to 3 weeks should be added to the test phase.</i>
Egg-laying exposure:	10 weeks	
Withdrawal period, if used:	6 weeks	

Parameter	Details	Remarks
		Criteria
<u>Pen (for parental and offspring)</u> Size: Construction materials: Number:	Parental: 25 x 51 x 20-26 cm (sloping floors). Offspring: 72 x 90 x 23 cm Galvanized wire mesh and galvanized sheeting. 16 parental pens/treatment level	<u>Pens</u> <i>Pens should have adequate room and be arranged to prevent cross-contamination.</i> <u>Materials</u> <i>Recommended materials include nontoxic material and nonbinding material, such as galvanized steel.</i> <u>Number</u> <i>At least 5 replicate pens should be used for mallards housed in groups of 7. For other arrangements, at least 12 pens should be used, but considerably more may be used if birds are kept in pairs. Chicks should be housed according to parental grouping.</i>
Number of birds per pen (male:female)	2 birds/pen (1 male:1 female)	<i>One male and one female per pen should be used. For quail, one male and two females should be used. For ducks, two males and five females should be used.</i>
<u>Number of pens per group/treatment</u> Negative control: Solvent control: Treated:	16 pens N/A 16 pens/treatment	<i>At least 12-16 pens should be used, but considerably more if birds are kept in pairs.</i>

Parameter	Details	Remarks
		Criteria
<u>Test concentrations (mg ai/kg diet)</u> Nominal: Measured:	0 (control), 20, 100, and 400 mg ai/kg diet <1.0 (<LOQ, control), 18.2, 100, and 381 mg ai/kg diet	<i>Recommended test concentrations include at least two concentrations other than the control; three or more will provide a better statistical analysis. The highest test concentrations should show a significant effect or be at or above the actual or expected field residue level.</i>
Maximum labeled field residue anticipated and source of information:	Not specified	<i>The highest test concentrations should show a significant effect or be at or above the actual or expected field residue level. The source (i.e., maximum label rate in lb ai/A and ppm), label registration no., label date, and site should be cited]</i>
<u>Solvent/vehicle, if used</u> Type: Amount:	None N/A	<i>Recommended solvents include corn oil or other appropriate vehicle not more than 2% of diet by weight</i>
Was detailed description and nutrient analysis of the basal diet provided? (Yes/No)	Yes	<i>A commercial breeder feed or an equivalent that is appropriate for the test species is recommended.</i>
Preparation of test diet	For each level, test substance was mixed into a premix basal feed. Test diets were prepared weekly beginning August 2, 2011 and presented to birds on Tuesday of each week.	<i>A premixed diet containing the test substance should be mechanically mixed with basal diet. If an evaporative vehicle is used, it should be completely evaporated prior to feeding.</i>
Indicate whether stability and homogeneity of test material in diet determined (Yes/No)	Yes	See Reviewer's Comments section.
Were concentrations in diet verified by chemical analysis?	Yes	See Reviewer's Comments section.

Parameter	Details	Remarks
		Criteria
Did chemical analysis confirm that diet was stable?	Yes	See Reviewer's Comments section.
Homogeneous?	Yes	
Feeding and husbandry	Feeding and husbandry conditions appeared to be adequate, given guideline recommendations.	
<u>Test conditions (pre-laying)</u> Temperature: Relative humidity: Photoperiod:	21.4 ± 0.7°C 57 ± 17% 8 hr light/day through Week 7; 17 hr light/day thereafter	Light intensity was 133 lux. <i>Recommended temperature: about 21°C (70°F)</i> <i>Recommended relative humidity: about 55%</i> <i>Recommended lighting</i> <i>First 8 weeks: 7 h per day.</i> <i>Thereafter: 16-17 h per day.</i> <i>At least 6 foot-candles are recommended at bird level.</i>
Egg Collection and Incubation		
<u>Egg collection and storage</u> Collection interval: Storage temperature: Storage humidity:	Daily 14.2 ± 0.1°C 85 ± 5%	<i>Eggs should be collected daily; recommended egg storage temperature is approximately 16°C (61°F); recommended humidity is approximately 65%.</i> <i>Recommended collection interval: daily</i>
Were eggs candled for cracks prior to setting for incubation?	Yes	<i>Eggs should be candled on day 0</i>
Were eggs set weekly?	Yes	
When candling was done for fertility?	Eggs were candled again on Days 11-12 (embryo viability) and 21 (embryo survival).	850.2300 also recommends candling on day 18 for Bobwhite quail <i>Quail: approx. day 11</i> <i>Ducks: approx. day 14</i>
When the eggs were transferred to the hatcher?	Day 21 eggs were transferred to Nature Form Incubator – no rotation after transfer—wire basket separation by pen of origin	<i>Bobwhite: usually day 21</i> <i>Mallard: usually day 23</i>

Parameter	Details	Remarks
		Criteria
<u>Hatching conditions</u> Temperature: Humidity: Photoperiod:	37.3 ± 0.0°C 57 ± 1% 17 hours light/day	<i>Recommended temperature is 39°C (102°F) Recommended humidity is 70%</i>
Day the hatched eggs were removed and counted	Day 25-26	<i>Eggs for Bobwhite should be removed on day 24; for mallard on day 27</i>
Were egg shells washed and dried for at least 48 hrs before measuring?	Yes-air dried for 1 week	
<u>Egg shell thickness</u> No. of eggs used: Intervals: Mode of measurement:	One egg from each of the odd numbered pens during odd numbered weeks and one egg from each of the even numbered pens during even numbered weeks were collected and measured. Weekly Five points around the waist of the egg using a micrometer to the nearest 0.002 mm.	<i>Newly hatched eggs should be collected at least once every two weeks. Thickness of the shell plus membrane should be measured to the nearest 0.01 mm with 3 - 4 measurements per shell.</i>
Reference chemical, if used	None used.	

2. Observations:

Table 3: Observations

Parameter	Details	Remarks
Parameters measured		
<u>Parental</u> (mortality, body weight, mean feed consumption) <u>Egg collection and subsequent development</u> (no. of eggs laid, no. of eggs cracked, shell thickness, no. of eggs set, no. of viable embryos, no. of live 3 week embryos, no. hatched, no. of 14-day survivors, average weight of 14-d old survivors, mortality, gross pathology, others)	- mortality - signs of toxicity - body weight - feed consumption - eggs laid per female/ maximum laid any female converted to percentage value - eggs cracked - egg shell thickness - eggs set - viable embryos-day 11-12 - live 3-week embryos of viable/pen - hatchlings of live embryos/pen - hatchlings/eggs set and maximum set - hatchling body weight - 14-day-old survivors of hatchlings/pen - 14 day survivors/eggs set - 14-day-old survivor body weight by parental pen group	<i>Recommended endpoints measured include:</i> <ul style="list-style-type: none"> Eggs laid/pen Eggs cracked/pen Eggs set/pen Viable embryos/pen Live 3-week embryos/pen Normal hatchlings/pen 14-day-old survivors/pen 14-day-old survivors/pen Weights of 14-day-old survivors (mean per pen) Egg shell thickness Food consumption (mean per pen) Initial and final body weight (mean per pen)
Indicate if the test material was regurgitated	No indications of dietary regurgitation.	
Observation intervals (for various parameters)	Parental mortality and signs of toxicity were recorded daily. Parental body weights were recorded on Weeks 0, 2, 4, 6, 8, and at adult termination. Offspring were weighed when removed from the hatcher and at 14 days. Parental feed consumption was measured weekly.	<i>Body weights and food consumption should be measured at least biweekly</i>
Were raw data included?	Yes	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

No mortalities occurred in the control or 400 mg ai/kg groups. One male bird in the 20 mg ai/kg group was found dead on Day 3 of Week 6, and one female bird in the 100 mg ai/kg group was found dead on Day 6 of

Week 20. These mortalities were considered incidental due to the nature of lesions observed at necropsy.

B. REPRODUCTIVE AND OTHER ENDPOINTS:

Abnormal Effects/Behavior: No treatment-related signs of toxicity were observed during the study. The incidental clinical observations of feather loss, lesions (head, foot, hock, leg and wing), abrasions, swelling, bruising, coughing, wing droop, lameness, thin appearance, and ventral head curl were associated with injuries and pen wear. All other birds were normal in appearance and behavior. No treatment-related findings were observed in the ≤ 100 mg ai/kg diet groups based on the gross necropsy results. At 400 mg ai/kg, the treatment-related finding of enlarged thyroids was observed for one male and four female adult birds.

Food Consumption: No significant treatment-related differences in food consumption were observed during the study. The estimated overall mean dietary doses were 0.0, 1.8, 8.9, and 35.3 mg ai/kg/day for the control, 20, 100, and 400 mg ai/kg groups, respectively.

Body Weight: No significant treatment-related differences were observed between the control group and any of the treatment groups at any of the adult body weight intervals. No treatment-related effects on offspring body weight were observed in the ≤ 20 mg ai/kg diet groups. However, at 100 and 400 mg ai/kg diet, hatchling body weights were significantly ($p < 0.05$) decreased compared to controls. Additionally at 400 mg ai/kg diet, 14-day old survivor weight was significantly ($p < 0.05$) reduced compared to controls.

Reproductive Effects: No statistically significant differences from controls were observed in any reproductive parameter in the ≤ 100 mg ai/kg diet groups. At 400 mg ai/kg diet, the live 3-week embryos as a percentage of viable embryos was significantly ($p < 0.01$) lower compared to the control. Also, at 400 mg ai/kg diet, slight reductions were observed in viable embryos per eggs set and hatchability, hatchlings per eggs set, and 14-day old survivors per eggs set. Although these reductions were not statistically significant, the authors considered it to be related to treatment.

No significant treatment-related differences in egg shell thickness were observed between the control group and any of the treatment groups.

Table 4: Reproductive and Other Parameters (nominal concentrations; study author-reported).

Parameter	Control	20 mg ai/kg	100 mg ai/kg	400 mg ai/kg	NOAEC/ LOAEC
No. laying pairs	16	15	16	16	400 mg ai/kg >400 mg ai/kg
Eggs laid/pair	818	737	865	823	400 mg ai/kg >400 mg ai/kg
Eggs cracked/pair	16	32	22	24	400 mg ai/kg >400 mg ai/kg
Eggs set/pair	726	637	761	700	400 mg ai/kg >400 mg ai/kg
Viable embryos/pair	624	618	698	549	400 mg ai/kg

Parameter	Control	20 mg ai/kg	100 mg ai/kg	400 mg ai/kg	NOAEC/ LOAEC
					>400 mg ai/kg
Live 3-week embryos/pair	622	615	694	536	400 mg ai/kg >400 mg ai/kg
Hatchlings/pair	567	570	610	461	400 mg ai/kg >400 mg ai/kg
14-day old hatchling survivors/pair	536	541	584	430	400 mg ai/kg >400 mg ai/kg
Eggs laid/hen	51	49	54	51	400 mg ai/kg >400 mg ai/kg
Eggs laid/hen/day (91 days)	0.56	0.54	0.59	0.57	400 mg ai/kg >400 mg ai/kg
14-day old survivors/hen	34	36	37	27	400 mg ai/kg >400 mg ai/kg
Eggs laid/maximum laid (%)	72	69	76	72	400 mg ai/kg >400 mg ai/kg
Eggs cracked/eggs laid (%)	2	5	3	3	400 mg ai/kg >400 mg ai/kg
Viable embryos/eggs set (%)	86	97	92	77 ^b	100 mg ai/kg 400 mg ai/kg
Live 3-week embryos/viable embryos (%)	100	100	100	98**	100 mg ai/kg 400 mg ai/kg
Hatchlings/Live 3-week embryos (%)	92	93	88	82	400 mg ai/kg >400 mg ai/kg
14-day old survivors/Hatchlings (%)	95	95	96	90	400 mg ai/kg >400 mg ai/kg
Hatchlings/Eggs set (%)	78	90	81	64 ^b	100 mg ai/kg 400 mg ai/kg
14-Day old survivors/Eggs set (%)	74	86	78	59 ^b	100 mg ai/kg 400 mg ai/kg
Hatchlings/Maximum set (%)	59	63	64	48	400 mg ai/kg >400 mg ai/kg
14-Day old survivors/Maximum set (%)	56	60	61	45	400 mg ai/kg >400 mg ai/kg

Parameter	Control	20 mg ai/kg	100 mg ai/kg	400 mg ai/kg	NOAEC/ LOAEC
Shell thickness (mm \pm SD)	0.234 \pm 0.012	0.229 \pm 0.010	0.228 \pm 0.015	0.234 \pm 0.020	400 mg ai/kg >400 mg ai/kg
Hatchling weight (g \pm SD)	6.2 \pm 0.4	6.1 \pm 0.4	5.8 \pm 0.4*	5.4 \pm 0.5**	20 mg ai/kg 100 mg ai/kg
14-day old survivors weight (g \pm SD)	28.8 \pm 2.2	28.0 \pm 2.6	28.1 \pm 2.4	26.3 \pm 3.6*	100 mg ai/kg 400 mg ai/kg
Mean food consumption (g/bird/day)	18	19	19	18	400 mg ai/kg >400 mg ai/kg
Weight (g) of parent females at test initiation: at Week 4: at test termination:	201 208 245	202 207 241	200 206 246	200 207 231	400 mg ai/kg >400 mg ai/kg
Weight (g) of parent males at test initiation: at Week 4: at test termination:	198 203 213	201 208 218	199 206 213	201 207 200	400 mg ai/kg >400 mg ai/kg

^a Data obtained from Page 23, Table 1 on page 26, Table 3 on page 31, Table 4 on page 33, Table 5 on page 35, and Table 6 on page 36 of the study report.

^b Treatment-related reductions were observed at 400 mg ai/kg, but not statistically significant.

* Statistically different from the control at $p < 0.05$.

** Statistically different from the control at $p < 0.01$.

C. REPORTED STATISTICS:

Each of the treatment groups was compared to the control group using an analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Procedure. Sample units were the individual pens within each experimental group, except adult body weights, where the sample unit was the individual bird. Percentage data were arcsine square root transformed prior to analysis. Nominal concentrations were used for all analyses.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Data were tested to determine if they satisfied the assumptions of normality using Shapiro-Wilks test and homogeneity of variances using Bartlett's test. Eggshell thickness, food consumption, and male weight gain met these assumptions of parametric statistics and was therefore analyzed using Dunnett's multiple comparison test. A suggestive decreasing monotonic response was noted for 14-d survivor weight, hatchling weight, and hatchlings per live embryo; the responses were not linear, though overall decreasing trends were exhibited. The Jonckheere-Terpstra Step-Down Test was used to analyze hatchlings per live embryo and Williams test was used to analyze hatchling weight and 14-d survivor weight. The Wilcoxon test with Bonferroni Adj was used to determine the NOAEL/LOAEL values for all endpoints which were non-parametric data that did not exhibit a monotonic response. Unless otherwise indicated, effects were considered statistically significant at $p < 0.05$. These analyses were conducted using CETIS version 1.8.7.12 with backend settings approved for use by

EFED on 5/29/13. Analyses were conducted using mean-measured concentrations.

NOAEL: 18.2 mg ai/kg

LOAEL: 100 mg ai/kg

Endpoints affected: 14-d hatchlings/eggs set, hatchlings/live embryo, hatchling weight, 14-d survivor weight, male weight gain, female weight gain

Most sensitive endpoint(s): Hatchling body weight

Table 5: Reproductive and Other Parameters (mean-measured concentrations; reviewer-reported).

Parameter	Control	18.2 mg ai/kg	100 mg ai/kg	381 mg ai/kg	NOAEC/ LOAEC
Eggs laid/pen	51.1	49.1	54.1	51.4	381 mg ai/kg >381 mg ai/kg
Eggs not cracked/eggs laid (%)	98.1	94.8	97.1	97.2	381 mg ai/kg >381 mg ai/kg
Shell thickness	0.234	0.229	0.228	0.234	381 mg ai/kg >381 mg ai/kg
Viable embryos/eggs set (%)	85.9	96.6	92.3	77.0	381 mg ai/kg >381 mg ai/kg
Live embryos/eggs set (%)	85.6	96.2	91.8	75.2	381 mg ai/kg >381 mg ai/kg
No. of hatchlings/eggs set (%)	78.4	89.7	80.9	64.2	381 mg ai/kg >381 mg ai/kg
14 Day hatchlings/eggs set (%)	74.2	85.4	77.7	59.0*	100 mg ai/kg 381 mg ai/kg
Live embryos/viable embryos (%)	99.7	99.6	99.4	97.5	381 mg ai/kg >381 mg ai/kg
Hatchling/live embryos (%)	92.2	93.4	87.9	82.3*	100 mg ai/kg 381 mg ai/kg
14 Day hatchling/no. hatched (%)	94.5	95.2	95.5	90.1	381 mg ai/kg >381 mg ai/kg
Hatchling weight (g)	6.25	6.13	5.88*	5.47**	18.2 mg ai/kg 100 mg ai/kg
14 Day survivor weight (g)	28.9	28.1	28.1	26.4*	100 mg ai/kg 381 mg ai/kg
Mean food consumption (g/bird/day)	18.3	18.8	18.6	18.4	381 mg ai/kg >381 mg ai/kg

Male weight gain (g)	15.8	18.1	13.5	-1.37**	100 mg ai/kg 381 mg ai/kg
Female weight gain (g)	44.6	38.7	46.5	30.4*	100 mg ai/kg 381 mg ai/kg

* Statistically different from the control at $p < 0.05$.

** Statistically different from the control at $p < 0.01$.

E. STUDY DEFICIENCIES:

This study is scientifically sound and there were no study deficiencies.

No deviations from OCSPP guideline 850.2300 were noted.

F. REVIEWER'S COMMENTS:

The reviewer's results were comparable to those of the study authors with the exception that the reviewer reported all results based on the mean-measured concentrations rather than the nominal concentrations. Therefore, the reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

Though not statistically significant, there were suggesting dose-related decreases in viable embryos/egg set (10.3% reduction), live embryos/egg set (12.1% reduction), and hatchlings/egg set (18.0% reduction) relative to the negative control.

All validity requirements were met. Specifically, controls produced an average of thirty-four (34) 14-day old survivors per hen during the 10-week production phase (minimum of 12 chicks per pen during a 10-week production phase), the egg shell thickness of control eggs was 0.234 mm (minimum of 0.19 mm for Bobwhite), and adult control mortality was $< 10\%$.

Homogeneity, stability, and concentration verification samples were analyzed for Ethylenethiourea concentrations during the definitive study and were included in the study report. The coefficients of variation were 4.48-6.65% for the homogeneity samples. The stability samples at Day 7 were 91-99% of the Day 0 values. The test concentrations were 91-100% of nominal for the verification samples.

Experimental test dates were August 2, 2011-January 30, 2012.

G. CONCLUSIONS:

This study is scientifically sound and is acceptable for Agency risk assessments. No mortalities occurred in the control or 381 mg ai/kg groups. Incidental mortalities included one 18.2 mg ai/kg group male bird found dead on Day 3 of Week 6, and one 100 mg ai/kg group female bird found dead on Day 6 of Week 20. No treatment-related signs of toxicity were observed in the control or treatment groups. No treatment-related findings were observed in the ≤ 100 mg ai/kg diet groups based on the gross necropsy results. At 381 mg ai/kg, the treatment-related finding of enlarged thyroids was observed for one male and four female adult birds. There were no treatment-related differences in feed consumption or in adult body weights at any treatment level. At 100 and 381 mg ai/kg diet, hatchling body weights were significantly ($p < 0.05$) decreased compared to controls. At 381

mg ai/kg diet, 14-day old survivor weight, 14-d hatchlings per egg set, hatchlings per live embryo, male weight gain, and female weight gain were significantly ($p < 0.05$) reduced compared to controls. No apparent treatment-related effects on reproduction or statistically-significant differences from the control were observed at any interval for the 18.2 or 100 mg ai/kg-diet levels. No significant treatment-related differences in egg shell thickness were observed between the control group and any of the treatment groups. The NOAEC was 18.2 mg ai/kg diet (mean-measured concentration) based on hatchling bodyweights.

NOAEC: 18.2 mg ai/kg (mean-measured)

LOAEC: 100 mg ai/kg (mean-measured)

Endpoints affected: 14-d hatchlings/eggs set, hatchlings/live embryo, hatchling weight, 14-d survivor weight, male weight gain, female weight gain

Most sensitive endpoint(s): Hatchling body weight

III. REFERENCES:

None; other than standard guidelines and methodologies.

CETIS Analytical Report

Report Date: 29 Dec-13 10:41 (p 1 of 18)

Test Code: 600016 48819701 | 02-5135-8581

OCSPP 850.2300 Chronic Avian Reproduction

Wildlife International

Analysis ID: 19-3871-3544

Analyzed: 29 Dec-13 10:38

Endpoint: 01 Eggs Laid per Pen

CETIS Version: CETISv1.8.7

Analysis: Nonparametric-Multiple Comparison

Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	20.1%	381	>381	NA	

Wilcoxon/Bonferroni Adj Test

Control	vs	C-mg ai/kgBW	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α :5%)
Negative Control		18.2	0.515	1.64	8	29	0.9098	Asymp	Non-Significant Effect
		100	-1.76	1.64	5	30	1.0000	Asymp	Non-Significant Effect
		381	-0.774	1.64	4	30	1.0000	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	191.356	63.78532	3	0.357	0.7840	Non-Significant Effect
Error	10532.36	178.5145	59			
Total	10723.71		62			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Bartlett Equality of Variance	5.56	11.3	0.1351	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.825	0.948	<0.0001	Non-normal Distribution

01 Eggs Laid per Pen Summary

C-mg ai/kgBW	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	16	51.1	46.2	56	52	29	64	2.29	17.9%	0.0%
18.2		15	49.1	42.7	55.6	50	14	65	3.01	23.7%	3.9%
100		16	54.1	46.2	62	59	9	70	3.7	27.4%	-5.75%
381		16	51.4	42.7	60.2	55	6	71	4.12	32.0%	-0.61%

Graphics

Batch ID:	19-8755-2466	Test Type:	Chronic Avian Repro	Analyst:	
Start Date:	02 Aug-11	Protocol:	OCSPP 850.2300 Chronic Bird	Diluent:	Not Applicable
Ending Date:		Species:	Colinus virginianus	Brine:	Not Applicable
Duration:	NA	Source:	Molly Bryden, Rock Hall, MD	Age:	20wk

CETIS Analytical Report

Report Date: 29 Dec-13 10:41 (p 2 of 18)

Test Code: 600016 48819701 | 02-5135-8581

OCSPP 850.2300 Chronic Avian Reproduction
International

Wildlife

Analysis ID: 10-7202-5659

Analyzed: 29 Dec-13 10:38

Endpoint: 02 Eggs Not Cracked per EL

CETIS Version: CETISv1.8.7

Analysis: Nonparametric-Multiple Comparison

Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	3.35%	<18.2	18.2	NA	

Wilcoxon/Bonferroni Adj Test

Control	vs	C-mg ai/kgBW	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α :5%)
Negative Control		18.2*	2.25	1.64	2	29	0.0365	Asymp	Significant Effect
		100	0.365	1.64	1	30	1.0000	Asymp	Non-Significant Effect
		381	0.527	1.64	3	30	0.8975	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	0.008880854	0.002960285	3	1.63	0.1927	Non-Significant Effect
Error	0.1073214	0.001819006	59			
Total	0.1162022		62			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Bartlett Equality of Variance	7.9	11.3	0.0481	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.753	0.948	<0.0001	Non-normal Distribution

02 Eggs Not Cracked per EL Summary

C-mg ai/kgBW	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	16	0.981	0.967	0.995	0.983	0.891	1	0.00665	2.71%	0.0%
18.2		15	0.948	0.918	0.979	0.962	0.786	1	0.0143	5.83%	3.32%
100		16	0.971	0.951	0.991	0.983	0.883	1	0.00926	3.82%	1.02%
381		16	0.972	0.947	0.998	0.981	0.807	1	0.0118	4.85%	0.87%

Graphics

Batch ID:	19-8755-2466	Test Type:	Chronic Avian Repro	Analyst:	
Start Date:	02 Aug-11	Protocol:	OCSPP 850.2300 Chronic Bird	Diluent:	Not Applicable
Ending Date:		Species:	Colinus virginianus	Brine:	Not Applicable
Duration:	NA	Source:	Molly Bryden, Rock Hall, MD	Age:	20wk

CETIS Analytical Report

OCSP 850.2300 Chronic Avian Reproduction

Wildlife International

Analysis ID: 15-6103-3578

Analyzed: 29 Dec-13 10:38

Endpoint: 03 Viable Embryos per ES

CETIS Version: CETISv1.8.7

Analysis: Nonparametric-Multiple Comparison

Official Results: Yes

Batch ID: 19-8755-2466

Test Type: Chronic Avian Repro

Analyst:

Start Date: 02 Aug-11

Protocol: OCSP 850.2300 Chronic Bird

Diluent: Not Applicable

Ending Date: Duration: NA

Species: Colinus virginianus

Source: Molly Bryden, Rock Hall, MD

Brine: Not Applicable

Age: 20wk

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	17.0%	381	>381	NA	

Wilcoxon/Bonferroni Adj Test

Control	vs	C-mg ai/kgBW	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α :5%)
Negative Control		18.2	-1.2	1.64	3	29	1.0000	Asymp	Non-Significant Effect
		100	0.246	1.64	1	30	1.0000	Asymp	Non-Significant Effect
		381	1.63	1.64	1	30	0.1560	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	0.3408882	0.1136294	3	3.18	0.0305	Significant Effect
Error	2.109224	0.03574957	59			
Total	2.450113		62			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Bartlett Equality of Variance	64.6	11.3	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.683	0.948	<0.0001	Non-normal Distribution

03 Viable Embryos per ES Summary

C-mg ai/kgBW	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	16	0.859	0.728	0.989	0.947	0.0213	1	0.0614	28.6%	0.0%
18.2		15	0.966	0.947	0.985	0.976	0.875	1	0.00878	3.52%	-12.5%
100		16	0.923	0.894	0.952	0.913	0.796	1	0.0135	5.85%	-7.53%
381		16	0.77	0.623	0.918	0.857	0	1	0.0691	35.9%	10.3%

OCSP 850.2300 Chronic Avian Reproduction

Wildlife International

Analysis ID: 10-5203-0165

Analyzed: 29 Dec-13 10:38

Endpoint: 04 Live Embryos per ES

CETIS Version: CETISv1.8.7

Analysis: Nonparametric-Multiple Comparison

Official Results: Yes

Batch ID:	19-8755-2466	Test Type:	Chronic Avian Repro		Analyst:					
Start Date:	02 Aug-11	Protocol:	OCSP 850.2300 Chronic Bird		Diluent:	Not Applicable				
Ending Date:		Species:	Colinus virginianus		Brine:	Not Applicable				
Duration:	NA	Source:	Molly Bryden, Rock Hall, MD		Age:	20wk				
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU	
Untransformed	NA	C > T	NA	NA	16.9%	381	>381	NA		

Wilcoxon/Bonferroni Adj Test

Control	vs	C-mg ai/kgBW	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		18.2	-1.09	1.64	3	29	1.0000	Asymp	Non-Significant Effect
		100	0.284	1.64	1	30	1.0000	Asymp	Non-Significant Effect
		381	1.91	1.64	1	30	0.0848	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.3902782	0.1300927	3	3.7	0.0165	Significant Effect
Error	2.07394	0.03515153	59			
Total	2.464218		62			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	62.8	11.3	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.687	0.948	<0.0001	Non-normal Distribution

04 Live Embryos per ES Summary

C-mg ai/kgBW	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	16	0.856	0.725	0.987	0.947	0.0213	1	0.0615	28.7%	0.0%
18.2		15	0.962	0.944	0.981	0.976	0.875	1	0.00861	3.46%	-12.4%
100		16	0.918	0.887	0.949	0.913	0.778	1	0.0144	6.28%	-7.28%
381		16	0.752	0.608	0.896	0.84	0	1	0.0677	36.0%	12.1%

OCSP 850.2300 Chronic Avian Reproduction

Wildlife International

Analysis ID: 01-9365-5745

Analyzed: 29 Dec-13 10:38

Endpoint: 05 Hatchlings per ES

CETIS Version: CETISv1.8.7

Analysis: Nonparametric-Multiple Comparison

Official Results: Yes

Batch ID:	19-8755-2466	Test Type:	Chronic Avian Repro			Analyst:			
Start Date:	02 Aug-11	Protocol:	OCSP 850.2300 Chronic Bird			Diluent:	Not Applicable		
Ending Date:		Species:	Colinus virginianus			Brine:	Not Applicable		
Duration:	NA	Source:	Molly Bryden, Rock Hall, MD			Age:	20wk		
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	20.6%	381	>381	NA	

Wilcoxon/Bonferroni Adj Test

Control	vs	C-mg ai/kgBW	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		18.2	-1.19	1.64	1	29	1.0000	Asymp	Non-Significant Effect
		100	0	1.64	1	30	1.0000	Asymp	Non-Significant Effect
		381	213	NA	0	30	0.0843	Exact	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.5232865	0.1744288	3	3.96	0.0122	Significant Effect
Error	2.596497	0.04400843	59			
Total	3.119784		62			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	21.3	11.3	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.841	0.948	<0.0001	Non-normal Distribution

05 Hatchlings per ES Summary

C-mg ai/kgBW	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	16	0.784	0.654	0.913	0.863	0.0213	1	0.0609	31.1%	0.0%
18.2		15	0.897	0.857	0.938	0.913	0.735	1	0.0189	8.14%	-14.5%
100		16	0.809	0.716	0.902	0.855	0.367	1	0.0437	21.6%	-3.21%
381		16	0.642	0.493	0.792	0.724	0	0.911	0.07	43.6%	18.0%

OCSP 850.2300 Chronic Avian Reproduction

Wildlife International

Analysis ID: 06-5967-9312

Analyzed: 29 Dec-13 10:36

Endpoint: 06 14d Hatchlings per ES

CETIS Version: CETISv1.8.7

Analysis: Nonparametric-Multiple Comparison

Official Results: Yes

Batch ID:	19-8755-2466	Test Type:	Chronic Avian Repro			Analyst:			
Start Date:	02 Aug-11	Protocol:	OCSP 850.2300 Chronic Bird			Diluent:	Not Applicable		
Ending Date:		Species:	Colinus virginianus			Brine:	Not Applicable		
Duration:	NA	Source:	Molly Bryden, Rock Hall, MD			Age:	20wk		
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	21.8%	100	381	195.2	

Wilcoxon/Bonferroni Adj Test

Control	vs	C-mg ai/kgBW	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		18.2	-1.11	1.64	1	29	1.0000	Asymp	Non-Significant Effect
		100	268	NA	0	30	1.0000	Exact	Non-Significant Effect
		381*	206	NA	0	30	0.0432	Exact	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.5786637	0.1928879	3	4.37	0.0076	Significant Effect
Error	2.603755	0.04413143	59			
Total	3.182418		62			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	18.3	11.3	0.0004	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.872	0.948	<0.0001	Non-normal Distribution

06 14d Hatchlings per ES Summary

C-mg ai/kgBW	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	16	0.742	0.61	0.873	0.838	0.0213	0.969	0.0617	33.3%	0.0%
18.2		15	0.854	0.811	0.898	0.868	0.673	0.95	0.0205	9.28%	-15.2%
100		16	0.777	0.678	0.875	0.824	0.317	1	0.0463	23.8%	-4.73%
381		16	0.59	0.446	0.733	0.638	0	0.867	0.0673	45.7%	20.5%

OCSP 850.2300 Chronic Avian Reproduction

Wildlife International

Analysis ID: 00-1835-0168

Analyzed: 29 Dec-13 10:35

Endpoint: 07 Live Embryos per VE

CETIS Version: CETISv1.8.7

Analysis: Nonparametric-Multiple Comparison

Official Results: Yes

Batch ID:	19-8755-2466	Test Type:	Chronic Avian Repro	Analyst:	
Start Date:	02 Aug-11	Protocol:	OCSP 850.2300 Chronic Bird	Diluent:	Not Applicable
Ending Date:		Species:	Colinus virginianus	Brine:	Not Applicable
Duration:	NA	Source:	Molly Bryden, Rock Hall, MD	Age:	20wk

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	1.45%	100	381	195.2	

Wilcoxon/Bonferroni Adj Test

Control	vs	C-mg ai/kgBW	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		18.2	0.401	1.64	1	29	1.0000	Asymp	Non-Significant Effect
		100	0.83	1.64	2	30	0.6096	Asymp	Non-Significant Effect
		381*	2.66	1.64	1	29	0.0118	Asymp	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.004722157	0.001574052	3	4.61	0.0058	Significant Effect
Error	0.01979126	0.0003412286	58			
Total	0.02451341		61			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	41.1	11.3	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.66	0.947	<0.0001	Non-normal Distribution

07 Live Embryos per VE Summary

C-mg ai/kgBW	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	16	0.997	0.991	1	1	0.967	1	0.0024	0.96%	0.0%
18.2		15	0.996	0.991	1	1	0.977	1	0.0022	0.86%	0.07%
100		16	0.994	0.989	1	1	0.976	1	0.00256	1.03%	0.23%
381		15	0.975	0.957	0.994	0.98	0.875	1	0.00868	3.45%	2.14%

OCSP 850.2300 Chronic Avian Reproduction

Wildlife International

Analysis ID: 12-2684-2431

Analyzed: 29 Dec-13 10:35

Endpoint: 08 Hatchlings per LE

CETIS Version: CETISv1.8.7

Analysis: Nonparametric-Multiple Comparison

Official Results: Yes

Batch ID:	19-8755-2466	Test Type:	Chronic Avian Repro			Analyst:				
Start Date:	02 Aug-11	Protocol:	OCSP 850.2300 Chronic Bird			Diluent:	Not Applicable			
Ending Date:		Species:	Colinus virginianus			Brine:	Not Applicable			
Duration:	NA	Source:	Molly Bryden, Rock Hall, MD			Age:	20wk			
Data Transform	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA		12.8%	381	>381	NA	

Wilcoxon/Bonferroni Adj Test

Control	vs	C-mg ai/kgBW	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		18.2	-0.219	1.64	3	29	1.0000	Asymp	Non-Significant Effect
		100	0.36	1.64	1	30	1.0000	Asymp	Non-Significant Effect
		381	1.8	1.64	1	29	0.1073	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1145901	0.03819671	3	1.69	0.1796	Non-Significant Effect
Error	1.31262	0.02263138	58			
Total	1.42721		61			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	14.3	11.3	0.0025	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.777	0.947	<0.0001	Non-normal Distribution

08 Hatchlings per LE Summary

C-mg ai/kgBW	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	16	0.922	0.864	0.98	0.956	0.596	1	0.0272	11.8%	0.0%
18.2		15	0.934	0.89	0.977	0.955	0.75	1	0.0203	8.44%	-1.22%
100		16	0.879	0.787	0.972	0.943	0.407	1	0.0434	19.8%	4.67%
381		15	0.823	0.709	0.937	0.902	0.214	1	0.0533	25.1%	10.8%

OCSP 850.2300 Chronic Avian Reproduction

Wildlife International

Analysis ID: 18-8379-6770

Analyzed: 29 Dec-13 10:34

Endpoint: 09 14d Hatchlings per NH

CETIS Version: CETISv1.8.7

Analysis: Nonparametric-Multiple Comparison

Official Results: Yes

Batch ID: 19-8755-2466 Test Type: Chronic Avian Repro Analyst:
 Start Date: 02 Aug-11 Protocol: OCSP 850.2300 Chronic Bird Diluent: Not Applicable
 Ending Date: Species: Colinus virginianus Brine: Not Applicable
 Duration: NA Source: Molly Bryden, Rock Hall, MD Age: 20wk

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	6.01%	381	>381	NA	

Wilcoxon/Bonferroni Adj Test

Control	vs	C-mg ai/kgBW	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		18.2	0.462	1.64	1	29	0.9658	Asymp	Non-Significant Effect
		100	0.174	1.64	2	30	1.0000	Asymp	Non-Significant Effect
		381	1.68	1.64	3	29	0.1390	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02899551	0.009665172	3	1.84	0.1503	Non-Significant Effect
Error	0.3050019	0.005258654	58			
Total	0.3339975		61			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	16.2	11.3	0.0010	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.793	0.947	<0.0001	Non-normal Distribution

09 14d Hatchlings per NH Summary

C-mg ai/kgBW	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	16	0.945	0.902	0.989	0.953	0.667	1	0.0204	8.64%	0.0%
18.2		15	0.952	0.929	0.975	0.951	0.868	1	0.0106	4.29%	-0.73%
100		16	0.955	0.931	0.979	0.955	0.864	1	0.0113	4.73%	-1.02%
381		15	0.901	0.843	0.958	0.931	0.667	1	0.0268	11.5%	4.69%

OCSP 850.2300 Chronic Avian Reproduction

Wildlife International

Analysis ID: 08-2300-2015

Analyzed: 29 Dec-13 10:34

Endpoint: 10 Eggshell Thickness

CETIS Version: CETISv1.8.7

Analysis: Parametric-Control vs Treatments

Official Results: Yes

Batch ID:	19-8755-2466	Test Type:	Chronic Avian Repro	Analyst:	
Start Date:	02 Aug-11	Protocol:	OCSP 850.2300 Chronic Bird	Diluent:	Not Applicable
Ending Date:		Species:	Colinus virginianus	Brine:	Not Applicable
Duration:	NA	Source:	Molly Bryden, Rock Hall, MD	Age:	20wk

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	4.71%	381	>381	NA	

Dunnett Multiple Comparison Test

Control	\ s	C-mg ai/kgBW	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		18.2	0.91	2.11	0.011	29	0.3627	CDF	Non-Significant Effect
		100	1.05	2.11	0.011	30	0.3047	CDF	Non-Significant Effect
		381	-0.132	2.11	0.011	30	0.7967	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0004869059	0.000162302	3	0.745	0.5298	Non-Significant Effect
Error	0.01286081	0.0002179798	59			
Total	0.01334771		62			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	7.19	11.3	0.0661	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.967	0.948	0.0896	Normal Distribution

10 Eggshell Thickness Summary

C-mg ai/kgBW	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	16	0.234	0.227	0.24	0.23	0.218	0.27	0.00314	5.37%	0.0%
18.2		15	0.229	0.223	0.234	0.229	0.205	0.243	0.00255	4.32%	2.07%
100		16	0.228	0.22	0.236	0.23	0.199	0.246	0.00368	6.46%	2.35%
381		16	0.234	0.224	0.245	0.231	0.208	0.283	0.00495	8.45%	-0.29%

OCSP 850.2300 Chronic Avian Reproduction

Wildlife International

Analysis ID: 03-1095-5044

Analyzed: 29 Dec-13 10:34

Endpoint: 11 Hatchling Weight

CETIS Version: CETISv1.8.7

Analysis: Parametric-Control vs Treatments

Official Results: Yes

Batch ID:	19-8755-2466	Test Type:	Chronic Avian Repro	Analyst:	
Start Date:	02 Aug-11	Protocol:	OCSP 850.2300 Chronic Bird	Diluent:	Not Applicable
Ending Date:		Species:	Colinus virginianus	Brine:	Not Applicable
Duration:	NA	Source:	Molly Bryden, Rock Hall, MD	Age:	20wk

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	6.79%	100	381	195.2	

Dunnett Multiple Comparison Test

Control	\ s	C-mg ai/kgBW	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		18.2	0.579	2.11	0.425	29	0.5096	CDF	Non-Significant Effect
		100	1.89	2.11	0.418	30	0.0780	CDF	Non-Significant Effect
		381*	3.89	2.11	0.425	29	0.0004	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.525269	1.841756	3	5.86	0.0014	Significant Effect
Error	18.21667	0.3140804	58			
Total	23.74194		61			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.29	11.3	0.5142	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.958	0.947	0.0318	Normal Distribution

11 Hatchling Weight Summary

C-mg ai/kgBW	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	16	6.25	6.01	6.49	6	6	7	0.112	7.16%	0.0%
18.2		15	6.13	5.85	6.42	6	5	7	0.133	8.42%	1.87%
100		16	5.88	5.55	6.2	6	5	7	0.155	10.5%	6.0%
381		15	5.47	5.11	5.82	6	4	6	0.165	11.7%	12.5%

OCSP 850.2300 Chronic Avian Reproduction

Wildlife International

Analysis ID: 06-0837-6420

Analyzed: 29 Dec-13 10:39

Endpoint: 11 Hatchling Weight

CETIS Version: CETISv1.8.7

Analysis: Parametric-Control vs Ord.Treatments

Official Results: Yes

Batch ID: 19-8755-2466 Test Type: Chronic Avian Repro Analyst:
 Start Date: 02 Aug-11 Protocol: OCSP 850.2300 Chronic Bird Diluent: Not Applicable
 Ending Date: Species: Colinus virginianus Brine: Not Applicable
 Duration: NA Source: Molly Bryden, Rock Hall, MD Age: 20wk

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	5.7%	18.2	100	42.66	

Williams Multiple Comparison Test

Control	\ s	C-mg ai/kgBW	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control	18.2		0.579	1.67	0.337	29	>0.05	CDF	Non-Significant Effect
	100*		1.89	1.75	0.346	30	<0.05	CDF	Significant Effect
	381*		3.89	1.77	0.356	29	<0.05	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.525269	1.841756	3	5.86	0.0014	Significant Effect
Error	18.21667	0.3140804	58			
Total	23.74194		61			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.29	11.3	0.5142	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.958	0.947	0.0318	Normal Distribution

11 Hatchling Weight Summary

C-mg ai/kgBW	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	16	6.25	6.01	6.49	6	6	7	0.112	7.16%	0.0%
18.2		15	6.13	5.85	6.42	6	5	7	0.133	8.42%	1.87%
100		16	5.88	5.55	6.2	6	5	7	0.155	10.5%	6.0%
381		15	5.47	5.11	5.82	6	4	6	0.165	11.7%	12.5%

OCSP 850.2300 Chronic Avian Reproduction

Wildlife International

Analysis ID: 18-8843-3135

Analyzed: 29 Dec-13 10:33

Endpoint: 12 14d Survivor Weight

CETIS Version: CETISv1.8.7

Analysis: Parametric-Control vs Treatments

Official Results: Yes

Batch ID: 19-8755-2466 Test Type: Chronic Avian Repro Analyst:
 Start Date: 02 Aug-11 Protocol: OCSP 850.2300 Chronic Bird Diluent: Not Applicable
 Ending Date: Species: Colinus virginianus Brine: Not Applicable
 Duration: NA Source: Molly Bryden, Rock Hall, MD Age: 20wk

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	7.37%	100	381	195.2	

Dunnett Multiple Comparison Test

Control	\ s	C-mg ai/kgBW	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		18.2	0.801	2.11	2.13	29	0.4106	CDF	Non-Significant Effect
		100	0.819	2.11	2.09	30	0.4030	CDF	Non-Significant Effect
		381*	2.45	2.11	2.13	29	0.0228	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	49.74691	16.5823	3	2.1	0.1096	Non-Significant Effect
Error	457.2208	7.883118	58			
Total	506.9677		61			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.53	11.3	0.2096	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.986	0.947	0.7201	Normal Distribution

12 14d Survivor Weight Summary

C-mg ai/kgBW	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	16	28.9	27.7	30	29.5	25	32	0.539	7.47%	0.0%
18.2		15	28.1	26.6	29.6	28	23	33	0.707	9.75%	2.8%
100		16	28.1	26.7	29.4	28	25	32	0.622	8.87%	2.81%
381		15	26.4	24.4	28.4	27	19	33	0.95	13.9%	8.57%

OCSP 850.2300 Chronic Avian Reproduction

Wildlife International

Analysis ID: 14-5381-7972

Analyzed: 29 Dec-13 10:39

Endpoint: 12 14d Survivor Weight

CETIS Version: CETISv1.8.7

Analysis: Parametric-Control vs Ord.Treatments

Official Results: Yes

Batch ID: 19-8755-2466 Test Type: Chronic Avian Repro Analyst:
 Start Date: 02 Aug-11 Protocol: OCSP 850.2300 Chronic Bird Diluent: Not Applicable
 Ending Date: Species: Colinus virginianus Brine: Not Applicable
 Duration: NA Source: Molly Bryden, Rock Hall, MD Age: 20wk

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	6.18%	100	381	195.2	

Williams Multiple Comparison Test

Control	\ s	C-mg ai/kgBW	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control	18.2		0.801	1.67	1.69	29	>0.05	CDF	Non-Significant Effect
	100		0.819	1.75	1.73	30	>0.05	CDF	Non-Significant Effect
	381*		2.45	1.77	1.78	29	<0.05	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	49.74691	16.5823	3	2.1	0.1096	Non-Significant Effect
Error	457.2208	7.883118	58			
Total	506.9677		61			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.53	11.3	0.2096	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.986	0.947	0.7201	Normal Distribution

12 14d Survivor Weight Summary

C-mg ai/kgBW	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	16	28.9	27.7	30	29.5	25	32	0.539	7.47%	0.0%
18.2		15	28.1	26.6	29.6	28	23	33	0.707	9.75%	2.8%
100		16	28.1	26.7	29.4	28	25	32	0.622	8.87%	2.81%
381		15	26.4	24.4	28.4	27	19	33	0.95	13.9%	8.57%

OCSP 850.2300 Chronic Avian Reproduction

Wildlife International

Analysis ID: 03-7020-4213

Analyzed: 29 Dec-13 10:33

Endpoint: 13 Mean Food Consumption (Adult)

CETIS Version: CETISv1.8.7

Analysis: Parametric-Control vs Treatments

Official Results: Yes

Batch ID:	19-8755-2466	Test Type:	Chronic Avian Repro			Analyst:			
Start Date:	02 Aug-11	Protocol:	OCSP 850.2300 Chronic Bird			Diluent:	Not Applicable		
Ending Date:		Species:	Colinus virginianus			Brine:	Not Applicable		
Duration:	NA	Source:	Molly Bryden, Rock Hall, MD			Age:	20wk		
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	5.0%	381	>381	NA	

Dunnett Multiple Comparison Test

Control	\ s	C-mg ai/kgBW	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control	18.2		-1.1	2.11	0.931	29	0.9735	CDF	Non-Significant Effect
	100		-0.718	2.11	0.916	30	0.9332	CDF	Non-Significant Effect
	381		-0.144	2.11	0.916	30	0.8005	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.376786	0.7922619	3	0.523	0.6680	Non-Significant Effect
Error	89.3375	1.514195	59			
Total	91.71429		62			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	7.68	11.3	0.0531	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.977	0.948	0.2813	Normal Distribution

13 Mean Food Consumption (Adult) Summary

C-mg ai/kgBW	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	16	18.3	17.8	18.9	18	16	20	0.254	5.54%	0.0%
18.2		15	18.8	18.4	19.2	19	18	20	0.2	4.12%	-2.66%
100		16	18.6	17.8	19.4	18	16	22	0.386	8.29%	-1.71%
381		16	18.4	17.6	19.1	18.5	16	21	0.352	7.66%	-0.34%

OCSPP 850.2300 Chronic Avian Reproduction

Wildlife International

Analysis ID: 10-8865-8087

Analyzed: 29 Dec-13 10:33

Endpoint: 14 Weight Gain MALE adult

CETIS Version: CETISv1.8.7

Analysis: Parametric-Control vs Treatments

Official Results: Yes

Batch ID:	19-8755-2466	Test Type:	Chronic Avian Repro			Analyst:				
Start Date:	02 Aug-11	Protocol:	OCSPP 850.2300 Chronic Bird			Diluent:	Not Applicable			
Ending Date:		Species:	Colinus virginianus			Brine:	Not Applicable			
Duration:	NA	Source:	Molly Bryden, Rock Hall, MD			Age:	20wk			
Data Transform	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA		62.1%	100	381	195.2	

Dunnett Multiple Comparison Test

Control	\ s	C-mg ai/kgBW	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		18.2	-0.489	2.11	9.99	29	0.8923	CDF	Non-Significant Effect
		100	0.496	2.11	9.83	30	0.5458	CDF	Non-Significant Effect
		381*	3.68	2.11	9.83	30	0.0007	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3673.349	1224.45	3	7.03	0.0004	Significant Effect
Error	10273.92	174.1342	59			
Total	13947.27		62			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.36	11.3	0.7156	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.984	0.948	0.5924	Normal Distribution

14 Weight Gain MALE adult Summary

C-mg ai/kgBW	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	16	15.8	8.8	22.8	16	-8	34	3.29	83.2%	0.0%
18.2		15	18.1	11.1	25.2	18	3	44	3.29	70.2%	-14.7%
100		16	13.5	7.48	19.5	13	-8	40	2.82	83.6%	14.6%
381		16	-1.37	-9.51	6.76	-1	-40	20	3.82	-1110.0%	109.0%

OCSP 850.2300 Chronic Avian Reproduction

Wildlife International

Analysis ID: 15-2044-7307

Analyzed: 29 Dec-13 10:32

Endpoint: 15 Weight Gain FEMALE adult

CETIS Version: CETISv1.8.7

Analysis: Nonparametric-Multiple Comparison

Official Results: Yes

Batch ID:	19-8755-2466	Test Type:	Chronic Avian Repro			Analyst:				
Start Date:	02 Aug-11	Protocol:	OCSP 850.2300 Chronic Bird			Diluent:	Not Applicable			
Ending Date:		Species:	Colinus virginianus			Brine:	Not Applicable			
Duration:	NA	Source:	Molly Bryden, Rock Hall, MD			Age:	20wk			
Data Transform	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA		32.1%	100	381	195.2	

Wilcoxon/Bonferroni Adj Test

Control	vs	C-mg ai/kgBW	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		18.2	0.435	1.64	3	29	0.9954	Asymp	Non-Significant Effect
		100	-0.455	1.64	2	29	1.0000	Asymp	Non-Significant Effect
		381*	2.49	1.64	4	30	0.0192	Asymp	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2447.394	815.7979	3	2.37	0.0798	Non-Significant Effect
Error	19964.54	344.2162	58			
Total	22411.94		61			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	11.2	11.3	0.0109	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.88	0.947	<0.0001	Non-normal Distribution

15 Weight Gain FEMALE adult Summary

C-mg ai/kgBW	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	16	44.6	39.7	49.5	45.5	31	63	2.3	20.6%	0.0%
18.2		15	38.7	26	51.5	43	-31	63	5.94	59.4%	13.1%
100		15	46.5	35.4	57.5	45	3	95	5.17	43.1%	-4.27%
381		16	30.4	20.1	40.8	35	-29	52	4.87	64.0%	31.7%